Eosinophilic glomerulonephritis in children in Southwestern Uganda

A Walker[1], J Ellis[1], M Irama[1], J Senkungu[1], D Nansera[1], J Axton[1], RJ Coward[3], DS Peat[2], HH Bode[1] and PW Mathieson[3]

[1]*Department of Paediatrics, Mbarara University of Science and Technology, Mbarara, Uganda,* [2]*Department of Histopathology, Norfolk and Norwich Hospital, Norwich, UK and* [3]*Academic Renal Unit, Department of Clinical Science of North Bristol, University of Bristol, Bristol, UK*

Acute renal disease is common in sub-Saharan Africa, with high mortality. Its etiology is poorly understood; quartan malaria owing to *Plasmodium malariae* was implicated in previous series. Few previous studies have included histological data; furthermore, much of the literature pre-dates the human immunodeficiency virus (HIV) epidemic. We report prospective analysis of acute proteinuric renal disease in children in rural Uganda. Clinical and laboratory data are presented on 65 patients (aged 2–14 years, mean 8.4; 35 male, 30 female) in 41 of whom histological diagnosis was obtained by renal biopsy. The most frequent histological finding was endocapillary proliferative glomerulonephritis (GN) in 27/41 cases, in 20 of which eosinophils were very prominent. No cases showed features of HIV nephropathy. Malarial films were positive in 11 cases: all owing to *Plasmodium falciparum*. Patients were treated with diuretics, antihypertensives, and supportive measures. Corticosteroids were rarely used, being reserved for patients with minimal changes on renal biopsy. Clinical outcomes were fair: 91% of patients survived to discharge. We conclude that acute GN is common in children in Uganda, that an unusual eosinophilic proliferative GN is the most frequent histological finding, that HIV is not implicated as an important factor in this age group, and that good outcomes can be achieved using simple clinical and laboratory diagnostic methods. Renal biopsy in selected cases is feasible and helpful, especially in allowing rational use of corticosteroids and other potentially toxic treatments. Symptomatic treatments and careful supportive care will allow the majority of children to recover.

Kidney International (2007) **71,** 569–573. doi:10.1038/sj.ki.5002085; published online 17 January 2007

KEYWORDS: proteinuria; childhood; malaria; HIV; Africa

Correspondence: *P Mathieson, Academic Renal Unit, Southmead Hospital, Bristol BS10 5NB, UK. E-mail: p.mathieson@bris.ac.uk*

Received 23 March 2006; revised 24 October 2006; accepted 21 November 2006; published online 17 January 2007

Old literature from Africa reported a high incidence of proteinuric renal disease in both children and adults: in the 1960s it accounted for 2–3% of medical admissions.[1] In general, a post-infectious etiology has been suspected in the majority of cases, with quartan malaria being implicated most frequently.[2,3] The advent of the human immunodeficiency virus (HIV) epidemic in sub-Saharan Africa introduced another important cause of proteinuric renal disease: HIV nephropathy is increasingly recognized in the developed world,[4] but renal manifestations of HIV have not been studied extensively in Africa. Two recent reports add more up-to-date information: Doe *et al.*[5] reported a retrospective analysis of 32 children in Ghana, including renal biopsy in 13 cases with focal segmental glomerulosclerosis being found in the majority of these. A letter in the New England Journal of Medicine[6] reports that in the Democratic Republic of Congo nephrotic syndrome accounts for 38% of admissions in the Nephrology Department and suggests that contemporary events in that country make it likely that the burden of renal disease will continue to increase. We have previously reported[7] a retrospective analysis of admissions to the children's ward in Mbarara University Hospital, Southwestern Uganda, in which we described an incidence of proteinuric renal disease that was at least eight times the UK incidence of childhood nephrotic syndrome. We found that quartan malaria was rare in Mbarara and that HIV was only apparently a factor in a minority of cases of children with proteinuric renal disease. We suggested[7] that a prospective study was required, including renal biopsy in selected cases, and we commenced such a study soon after our initial report. Here we report our results.

RESULTS

Information was recorded on 65 patients (35 male, 30 female). Age range was 2–14 years, mean 8.4. The main presenting complaint was body swelling in the vast majority of cases (59 body swelling, two with shortness of breath, two with cough, one with palpitations, one with knee pain). Of the 59 patients primarily presenting because of body swelling, on questioning 15 also complained of shortness of breath and five of cough. The duration of the illness ranged from 2 days

to 12 months, mean 36 days. Fourteen patients reported having been treated by a traditional healer with herbal medicines before attending the hospital.

On physical examination, all patients had periorbital and/or limb edema, 36 had ascites, five had gross pleural effusions, and five had signs of pericardial effusions. The majority were hypertensive, systolic blood pressures up to 210 mm Hg and diastolic pressures up to 155 mm Hg being recorded.

Urine dipstick testing showed heavy proteinuria (++ or greater) in 60/65 cases and heavy hematuria (++ blood or greater) in 60/65: rare individuals had only protein or blood in their initial urine sample. Urine microscopy showed red cell casts in 31 cases and granular casts in a further 20: only 14 of the 65 cases had neither. Anemia was almost universal: mean hemoglobin was 9.6 g/dl (range 6.4–14.3) with normochromic normocytic appearances in the vast majority, only two patients showing a microcytic appearance and one a megaloblastic appearance. Total white blood cell count was normal in all except five cases, peripheral blood eosinophilia was noted in only one case (25% eosinophils in total white cell count of 8.8×10^9/l), platelets were normal in all except four cases. Blood smears for malarial parasites were positive in 11 cases (two with + i.e., low-level parasitemia; eight with ++ i.e., moderate; one with +++ i.e., heavy, all *Plasmodium falciparum*). Excretory kidney function was impaired in the majority of cases: serum creatinine ranged from 0.6 to 12.1 mg/dl with mean of 3.0. Hypocomplementemia was frequent: serum complement C3 and C4 were measured in 62 cases at presentation: C3 was below normal in 55 cases and C4 was below normal in 55. Hypocomplementemia was often severe, especially for depression of serum C3. Anti-DNAse was tested in 64 cases and was positive in 32, anti-streptolysin O titer was tested in 63 cases and was elevated in 43. Discordance between these two tests for recent streptococcal infection (i.e. one test positive, the other negative) was common, being seen in 23 out of 62 for whom results of both were available. Renal ultrasound scan was performed in 48 cases: appearances were normal in 18, showed echogenic kidneys of normal or increased size in 28 and showed small kidneys in two.

Percutaneous renal biopsy was performed in 51 cases: in a further 10 cases renal biopsy was planned but not performed owing to patient being deemed too systemically unwell (four, all of whom subsequently died), lack of parental consent (two) withdrawal of consent when the patient's condition improved (one) and technical difficulties with equipment or facilities (three). Renal biopsy findings are summarized in Table 1. Adequate tissue for histological examination was obtained in 41 cases. Twenty-six of these showed diffuse proliferative glomerulonephritis (DPGN): intra-glomerular eosinophils were very prominent in 20 of the 26 (Figure 1). In these cases mean eosinophil count per glomerulus ranged from 4 to 8. One further case showed focal proliferative glomerulonephritis (GN); four showed advanced glomerulosclerosis compatible with end-stage kidney disease, with evidence of old crescentic GN in three of these; six were normal or showed only mild mesangial proliferation, these patients having heavy proteinuria so they were considered to have minimal change nephropathy; two showed focal necrotizing GN; one showed membranous GN and one showed focal segmental glomerulosclerosis. None of the patients had clinical features suggesting HIV infection and none of the renal biopsies had any features to suggest HIV nephropathy. In only one case (Figure 2) was malarial pigment seen in the renal biopsy. Immunohistology findings are summarized in Table 1: the majority of cases of DPGN showed granular C3 deposition (example shown in Figure 3), usually with associated immunoglobulin G deposition. No cases showed mesangial immunoglobulin A deposition. Electron microscopy was performed on re-processed tissue at a later date in selected cases with DPGN: electron-dense deposits were seen in sub-epithelial and/or intra-membranous location (example shown in Figure 4). Renal biopsy result was usually available within 7 days.

Regarding treatment, all patients received diuretics and antihypertensives as required, 46 received antibiotics (usually penicillin), 11 received anti-malarials (usually quinine), nine

Table 1 | Renal biopsy findings (41 adequate samples)

Diagnosis	Number of cases	Immunohistology
Eosinophilic diffuse proliferative GN	20	Eleven C3 and IgG positive Two C3 only One IgG only One IgM only Three negative Two not available
Diffuse proliferative GN	6	Two C3 and IgG positive Two C3 only One IgM only One negative
Focal proliferative GN	1	C3 and IgG positive
Normal or only mild mesangial proliferation	6	Six negative
End-stage glomerulosclerosis	4	Two negative Two weak C1q
Focal necrotizing GN	2	One negative One IgM only
Membranous GN	1	One IgG and C3 positive
Focal segmental glomerulosclerosis	1	One negative

GN, glomerulonephritis; Ig, immunoglobulin.

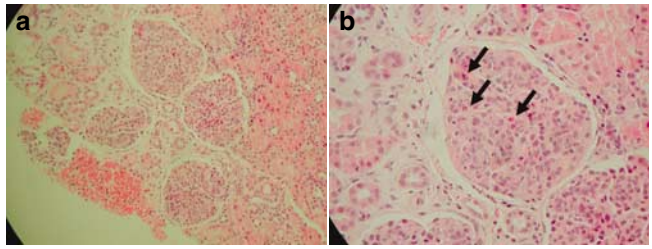

Figure 1 | Eosinophilic DPGN. (**a**) Renal biopsy showing the eosinophilic DPGN that was the most frequent finding in our series (hematoxylin and eosin stain). (**b**) One glomerulus from (**a**) in which some eosinophils are arrowed.

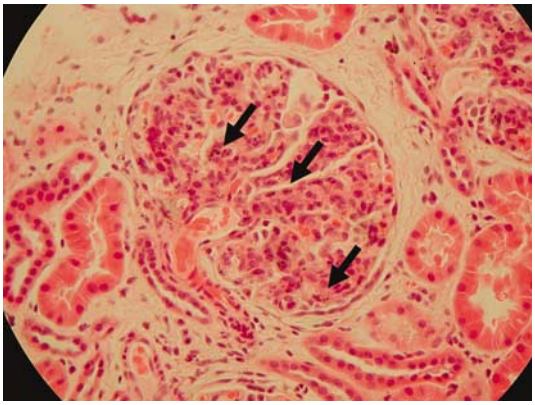

Figure 2 | Glomerulus from case of DPGN showing probable malaria pigment (arrowed).

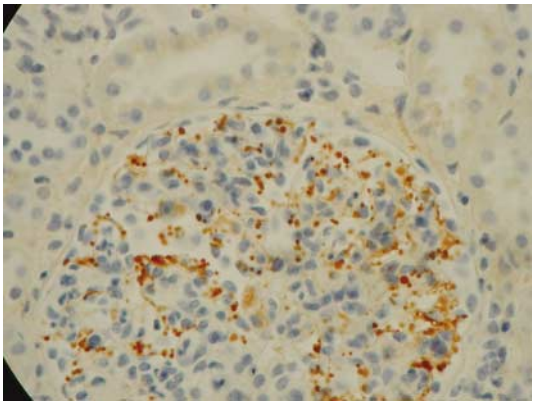

Figure 3 | Immunoperoxidase staining for C3 in DPGN.

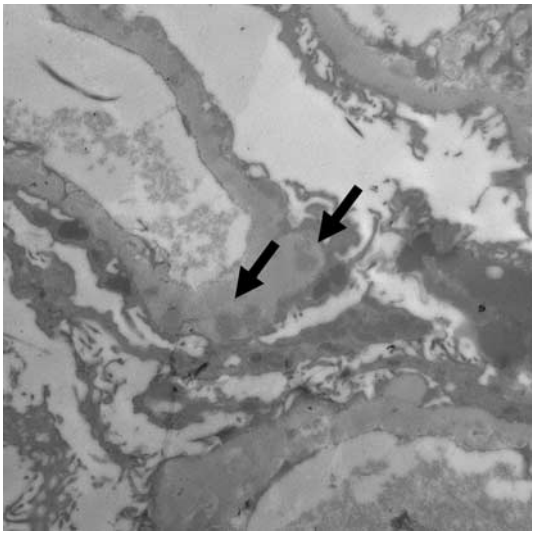

Figure 4 | Electron microscopy in DPGN showing subepithelial/intra-membranous electron dense deposits (arrowed).

received prednisolone. Four patients died in hospital (6% in-hospital mortality in the 65 patients); one patient was taken from the ward by the parents/self-discharged, one further patient was discharged in a comatose state and expected to die, so that 59 of the original 65 patients survived to elective discharge (91%, so that estimated overall mortality was 9%). Blood pressure had improved on treatment in all cases with initial hypertension. Serum creatinine was available at early follow-up (usually 7 days) in 41 patients and ranged from 0.3 to 12.5 mg/dl (mean 2.6). Unfortunately, longer-term follow-up is rarely possible in this setting.

DISCUSSION

The striking novel findings from this study were: (1) the predominance on renal biopsy of DPGN with prominent eosinophil infiltration; (2) an overall early mortality rate which was slightly lower than in our previous retrospective study (9% mortality compared to 17%)[7] but remains significant, especially as it might be preventable if better support facilities including short-term dialysis were available; (3) the feasibility of informing management of patients in rural Uganda by renal biopsies being transported to the UK for interpretation; (4) the utility or lack of it of various clinical parameters and simple laboratory tests as predictors of the underlying renal lesion and its prognosis.

The eosinophilic GN makes us suspect that one or more parasitic infections may underlie the condition: this will be an important subject for future study. Filariasis is locally prevalent and has been previously implicated in one similar case.[8] Unfortunately we were not able to obtain commercial antibodies to filarial antigens with which to analyze the renal tissue in this series. Dietary components can cause eosinophilic reactions[9] and represent another possible underlying cause, as do constituents of local traditional medicines. Malaria was not a prominent finding: 11 cases out of 65 (17%) had positive blood smears, all showing *Plasmodium falciparum*. Malaria is very common in Mbarara and this rate of positive blood smears is no higher than would be expected in acute unselected admissions to the hospital. We have reported [7] that *Plasmodium malariae*, the malaria species previously associated with acute nephritis in Africa, is rare in Mbarara. In only one case was malarial pigment seen in the renal biopsy (Figure 2). Nor did we find evidence that HIV is an important factor: none of the renal biopsies showed features of HIV nephropathy. The mean age of affected children (8.4 years) is against a role for HIV as vertically transmitted HIV infections are usually manifest at a younger age. Routine HIV testing is not considered ethical in Uganda without appropriate counselling and follow-up and was not undertaken on our patients. In designing our study we suspected that streptococcal infection might be important so we devised practical simple methods of assessing this using kits which require no specialized equipment or complex laboratory conditions. However, in most patients, measurement of serum C3, C4, anti-DNAse B, and ASO titer did not predict the findings on renal biopsy nor the prognosis. Abnormal results of these tests were very frequent, presumably reflecting a high prevalence of streptococcal and/or other infections in this young rural population. The best

predictor of acute proliferative GN on renal biopsy was the presence of red cell casts on urine microscopy (present in all cases of DPGN and also in two cases of end-stage scarring owing to previous GN, not present in those with steroid-responsive lesions). This study also confirmed observations made in our previous retrospective study: especially the lack of apparent association with malaria or HIV and the predominance of acute nephritic type presentations with hypertension, salt and water overload (edema, ascites, pleural effusions, and so on) and azotemia.

We believe that simple clinical management is responsible for the reasonable outcome in the present series. Increased awareness of acute nephritis, aggressive treatment with diuretics and antihypertensives, renal biopsy in selected cases, and selective use of corticosteroids for patients whose renal biopsy showed lesions likely to respond were all part of our practice during this period. Widespread use of antibiotics may have contributed to the good outcome but as we do not have a control group randomized not to receive antibiotics it is difficult to be certain of this.

We believe that renal biopsy in selected cases is practicable and helpful, but obviously it would be preferable to provide this service closer to the patients. Training of personnel in performance of renal biopsy and suitable after care of patients undergoing this procedure, availability of facilities for processing of biopsy tissue, and access to expert histopathological interpretation of renal biopsies all pose formidable problems for the developing world. In this series, renal biopsy was used to inform management. Eight patients with minimal change nephropathy or focal segmental glomerulosclerosis responded to prednisolone. One with focal necrotizing GN also showed some response to prednisolone: in the developed world such patients would also receive cytotoxic drug therapy as prednisolone alone is not considered adequate. Although empirical corticosteroid therapy is widely used in the developing world for children with nephritis, its efficacy is unproven. We have discontinued this practice: many of our patients with florid nephritis made excellent recoveries without corticosteroid treatment. Renal biopsies in four patients showed advanced renal scarring, possibly as a late result of previous nephritis, so that potentially toxic therapy would not be justified. Sadly, advanced kidney damage carries a poor prognosis in a health-care system where renal replacement therapy (dialysis or renal transplantation) is not yet widely available, but at least the renal biopsy findings allowed the parents to be informed of the prognosis. These four patients all had marked impairment of excretory renal function. However, it is noteworthy that other patients with equally severe renal impairment at presentation showed acute nephritis without scarring and made an excellent recovery. Thus the renal biopsy can give prognostic as well as diagnostic information that cannot be inferred from less invasive tests.

We believe that there is potentially preventable major morbidity and mortality owing to acute renal disease that make wider availability of histopathological diagnosis an important goal for the future. As acute nephritis may carry a good long-term prognosis if patients can be supported through the acute stages, we also believe that provision of short-term peritoneal dialysis for patients with severe azotemia and/or intractable salt and water overload should also be an important goal. Despite the logistical and financial problems that this poses, it is our ambition to establish this form of treatment in Mbarara.

In conclusion, renal biopsies in this series showed a surprising predominance of a novel eosinophilic GN whose etiology remains unexplained. Simple clinical parameters may be used to predict steroid-responsive lesions but more detailed understanding of the nature of GN, its prognosis and the best ways to design effective therapies for the future requires histological analysis. One important goal, recognized by the International Society of Nephrology, is to widen access to histopathological expertise in the developing world. Informed by studies such as ours, improved prevention, detection and treatment of renal disease can be attained so that the International Society of Nephrology mission[10] of 'preventive nephrology' can be supported whilst treatment of established renal failure remains a distant goal for many parts of the developing world.

MATERIALS AND METHODS

The study protocol was approved by the Ethics committee at Mbarara University Hospital. Consecutive admissions to the pediatric ward at Mbarara University Hospital in the period March 2001–April 2003 were considered for the study. Urine was tested for protein and blood: all children with proteinuria of 2+ or more and/or hematuria with or without a complaint of body swelling were considered to have possible nephritis and information was collected. Clinical details were recorded including age, presenting complaint, duration of illness, past medical history, and initial findings on clinical examination (especially blood pressure, edema, pleural effusions/ascites).

Investigations (which are severely limited in this resource-poor setting) included urine dipstick testing, blood films for malarial parasites, hematocrit, serum creatinine, serum complement C3 and C4 by radial immunoprecipitation assay using kits from The Binding Site (Birmingham, UK) according to the manufacturer's instructions; anti-DNAse and anti-streptolysin O titer using kits from bioMerieux (Basingstoke, UK) according to the manufacturer's instructions.

Renal ultrasound scan was performed in the majority of cases and percutaneous renal biopsy was undertaken in selected cases. Renal biopsy was not performed if the parent/carer did not give informed consent, if the child was deemed too systemically unwell, if the kidneys appeared small or inaccessible on ultrasound scan or for technical reasons (e.g. lack of assistants or equipment, lack of access to ultrasound facilities, and inadequate sedation). Renal biopsies were fixed in paraformaldehyde for transport to Norwich, UK by post and results relayed by e-mail back to Mbarara. Light microscopic and immunoperoxidase analyses used standard methodology. Later, fixed samples were re-processed for examination by electron microscopy. Follow-up information including treatment, clinical outcome, and findings on clinical examination and repeat urine and blood testing where possible was also recorded.

ACKNOWLEDGMENTS

We thank the children and their families, the medical and nursing staff of Mbarara University Hospital and Professor Eldryd Parry of the Tropical Health Education Trust, UK who has facilitated the link with University of Bristol, UK.

REFERENCES

1. Kibukamusoke JW. The nephrotic syndrome in Lagos, Nigeria. *W Afr Med J* 1966; **15**: 213–217.
2. Kibukamusoke JW, Hunt MSR, Wilks NE. The nephrotic syndrome in Uganda and its association with quartan malaria. *Q J Med* 1967; **143**: 393–408.
3. Hendrickse RG, Adeniyi A. Quartan malarial nephrotic syndrome in children. *Kidney Int* 1979; **16**: 64–74.
4. Winston JA, Burns GC, Klotman PE. The human immunodeficiency virus (HIV) epidemic and HIV-associated nephropathy. *Semin Nephrol* 1998; **18**: 373–377.
5. Doe JY, Funk M, Mengel M, Doehring E, Ehrich JH. Nephrotic syndrome in African children: lack of evidence for 'tropical nephrotic syndrome'? *Nephrol Dial Transplant* 2006; **21**: 672–676.
6. Pakasa NM, Sumaili EK. The nephrotic syndrome in the Democratic Republic of Congo. *New Engl J Med* 2006; **354**: 1085–1086.
7. Newsom DH, Bode HH, Kiwanuka J, Mathieson PW. Proteinuric renal disease in children in Southwestern Uganda. *Q J Med* 2003; **96**: 382–384.
8. Date A, Gunasekaran V, Kirubakaran MG, Shastry JC. Acute eosinophilic glomerulonephritis with Bancroftian filariasis. *Postgrad Med J* 1979; **55**: 905–907.
9. Varga J, Uitto J, Jimenez SA. The cause and pathogenesis of the eosinophilia-myalgia syndrome. *Ann Intern Med* 1992; **116**: 140–147.
10. Prevention of Renal Disease in the Emerging World. Toward Global Health Equity Proceedings of the Bellagio Conference, March 16–18, 2004, Italy. *Kidney Int* 2005; **98**(Suppl): S1–S94.